FORM PTO-1390 (REV 10-95)

U.S. DEPARTMENT OF COMMERCE AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: BHV-313.01

U S APPLICATION NO (If known, see 37 CFR 1.5): 09/402680

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AT98/00090	(06.04.98) 06 April 1998	(08.04.97) 08 April 1997

TITLE OF INVENTION: METHODS FOR INACTIVATING PATHOGENS IN A BIOLOGICAL MATERIAL

APPLICANT(S) FOR DO/EO/US
SCHWARZ, Hans-Peter, Gerol ZERLAUTH and TURECEK, Peter

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is the FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ( ) This a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. (X) This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l).
4. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. (X) A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. (X) is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ( ) has been transmitted by the International Bureau.
   c. ( ) is not required, as the application was filed in the United States Receiving Office (RO/US).
6. (X) A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. (X) Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. (X) are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ( ) has been transmitted by the International Bureau.
   c. ( ) have not been made; however, the time limit for making such amendments has NOT expired.
   d. ( ) have not been made and will not be made.
8. (X) A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. (X) An unexecuted oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ( ) A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ( ) An information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ( ) An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. (X) A FIRST preliminary amendment.
    ( ) A SECOND or SUBSEQUENT preliminary amendment.
14. ( ) A substitute specification.
15. ( ) A change of power of attorney and/or address letter.
16. (X) Other items of information.
    (X) Copy of PCT Request Form
    (X) Copy of Cover Sheet of international publication no. WO98/44941
    (X) Copy of International Preliminary Examination Report
    (X) Copy of International Search Report
    (X) Translation of International Search Report
    (X) Copy of Form PCT/IB/306 (Notification of the Recording of a Change - New Assignee is BAXTER AKTIENGESELLSCHAFT)
    (X) Copy of Form PCT/IB/332 (Information Concerning Elected Offices Notified of Their Election)
    (X) Copy of Form PCT/IB/308 (Notice Informing the Applicant of the Communication of the International Application to the Designated Offices)

Certificate of Express Mail

I hereby certify that the foregoing documents are being deposited with the United States Postal Service as Express Mail, postage prepaid, "Post Office to Addressee", in an envelope addressed to the Assistant Commissioner for Patents, Box PCT, Attn: DO/EO/US, Washington, D.C. 20231 on the date indicated below.

Ariel Collazo

Express Mail Label: EM528940143US
Date of Deposit: October 7, 1999

514 Rec'd PCT/PTO 07 OCT 1999

U.S. APPLICATION NO (if known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/402680	PCT/AT98/00090	BHV-313.01

17. (x) The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

	Search Report has been prepared by the EPO or JPO	$840.00
	International preliminary examination fee paid to USPTO (37 CFR 1.482)	$670.00
X	No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	$760.00
	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$970.00
	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	$ 96.00

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 970.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ( ) 20 (x) 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$0	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	34 - 20 =	14	X $18.00	$ 252.00	
Independent claims	2 - 3 =		X $78.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$0	
	TOTAL OF ABOVE CALCULATIONS =			$1222.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$0	
	SUBTOTAL =			$1222.00	
Processing fee of **$130.00** for furnishing the English translation later than ( ) 20 ( ) 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$0	
	TOTAL NATIONAL FEE =			$1222.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. +				$0	
	TOTAL FEES ENCLOSED =			$1222.00	
				Amount to be: refunded	$
				charged	$

a. (X) A check in the amount of $1222.00 to cover the above fees is enclosed.

b. ( ) Please charge my Deposit Account No **06-1448** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. (X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **06-1448, (Ref. BHV-313.01).** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Patent Group
Foley, Hoag & Eliot LLP
One Post Office Square
Boston, MA 02109-2170

SIGNATURE

Beth E. Arnold
REGISTRATION NO. 35,430

Attorney Docket No.: BHV-313.01

#3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Schwarz et al.

U.S. Application Serial No.: 09/402,680
National Phase of PCT/AT98/00090
Filed Apr. 6, 1998 (Priority Date: Apr. 8, 1997)

For: *A Method for Inactivating Pathogens, in Particular Viruses, in a Biological Material*

Filed: October 7, 1999

Attorney Docket No.: **BHV-313.01**

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December 9, 1999
Date of Signature and of Mail Deposit

By Carmen Parra
Carmen Parra

PRELIMINARY AMENDMENT

Dear Sir:

Please amend the above-identified patent application as follows:

In the title:

-- [A] Methods for Inactivating Pathogens[, in Particular Viruses,] in a Biological Material --

No new matter has been added to the application. Accordingly, it is respectfully requested that the above amendment be entered. Applicants submit that the changes being made in the preliminary amendment are in compliance with all patentability requirements.

Respectfully submitted,
Foley, Hoag & Eliot, LLP

Beth E. Arnold
Registration No. 35,430
Attorney for Applicant

Date: December 9, 1999

Patent Group
One Post Office Square
Boston, Massachusetts 02109
Telephone: (617) 832-1177
Facsimile: (617) 832-7000

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Schwarz et al.

U.S. Application Serial No.: To Be Determined;
National Phase of PCT/AT98/00090
Filed Apr. 6, 1998 (Priority Date: Apr. 8, 1997)

For: *Methods for Inactivating Pathogens in a Biological Material*

Filed: October 7, 1999

Attorney Docket No.:
BHV-313.01

Box PCT
Assistant Commissioner for Patents
U.S. Patent and Trademark Office
Washington, D.C. 20231

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October 7, 1999
Date of Signature and of Mail Deposit

By: _____
Ariel Collazo

PRELIMINARY AMENDMENT

Dear Sir:

Please amend the above-identified patent application as follows:

In the claims:

Cancel claims 1-8 and add new claims 9-42 as follows:

-- 9. A method for inactivating pathogens in a biological material by incubating for an appropriate period of time said biological material with a chemical agent, comprising adsorbing said biological material on a solid carrier and incubating with said chemical agent in the presence of an eluotropic salt corresponding to a NaCl concentration of at least 200 mM, incubation thereby being effected simultaneously with or immediately after elution of said biological material.

10. A method as set forth in claim 9, wherein said pathogens are viruses.

11. A method as set forth in claim 9, wherein said eluotropic salt corresponds to a NaCl concentration of at least 300 mM.

12. A method as set forth in claim 9, wherein said chemical agent is a detergent.

13. A method as set forth in claim 12, wherein said detergent is in an amount of at least 1%.

14. A method as set forth in claim 12, wherein said detergent is in an amount of more than 5%.

15. A method as set forth in claim 12, wherein said detergent is in an amount of more than 10%.

16. A method as set forth in claim 9, wherein said eluotropic salt is sodium chloride.

17. A method as set forth in claim 9, wherein said period of time for incubating is between 10 min and 10 h.

18. A method as set forth in claim 17, wherein said period of time for incubating is between 1 h and 5 h.

19. A method as set forth in claim 9, wherein said biological material is selected from the group consisting of plasma, a plasma fraction and a material from a cell culture.

20. A method as set forth in claim 9, wherein said biological material comprises a blood factor.

21. A method as set forth in claim 9, wherein said biological material comprises a vitamin K-dependent protein.

22. A method as set forth in claim 9, wherein said biological material is a prothrombin complex-containing fraction.

23. A method as set forth in claim 9, wherein said biological material adsorbed on said solid carrier is purified, and said incubation is effected after said elution of said purified material.

24. A method as set forth in claim 9, wherein said solid carrier is a chromatographic material.

25. A method as set forth in claim 24, wherein said chromatographic material is used in ion exchange chromatography or affinity chromatography.

26. A method as set forth in claim 9, further comprising an additional step for purifying said biological material.

27. A method as set forth in claim 26, wherein said additional step for purifying comprises a chromatographic purification.

28. A method as set forth in claim 9, further comprising an additional step of inactivating and/or depleting pathogens.

29. A method as set forth in claim 28, wherein said additional step is selected from the group consisting of a filtration and a heat treatment.

30. A method as set forth in claim 9, wherein said chemical agent is a non-ionic detergent selected from the group consisting of Tween and Triton.

31. A chromatographically purified preparation comprising an autodynamically activatable blood factor in a portion of less than 50%, based on its content of activated and non-activated blood factor, and a detergent content.

32. A preparation as set forth in claim 31, wherein said autodynamically activatable blood factor is comprised in a portion of less than 40%.

33. A preparation as set forth in claim 31, wherein said autodynamically activatable blood factor is comprised in a portion of less than 30%.

34. A preparation as set forth in claim 31, wherein said autodynamically activatable blood factor is comprised in a portion of less than 20%.

35. A preparation as set forth in claim 31, wherein said autodynamically activatable blood factor is comprised in a portion of less than 10%.

36. A preparation as set forth in claim 31, wherein said autodynamically activatable blood factor is comprised in a portion of less than 1%.

37. A preparation as set forth in claim 31, wherein said blood factor is selected from the group consisting of factor VII, factor XII, factor XI and prekallikrein.

38. A preparation as set forth in claim 31, said preparation comprising a prothrombin complex with a factor VIIa activity of less than 50%, based on its content of activated and non-activated factor VII.

39. A preparation as set forth in claim 38, wherein said factor VIIa activity is less than 10%.

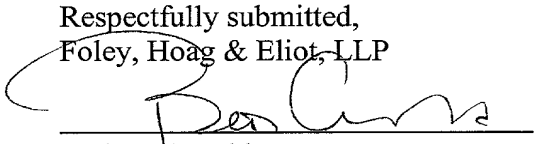
40. A preparation as set forth in claim 38, wherein said factor VIIa activity is less than 1%.

41. A preparation as set forth in claim 31, wherein said preparation is free from serine protease inhibitors and serine protease cofactors.

42. A preparation as set forth in claim 31, which is obtainable by a method for inactivating pathogens in a biological material by incubating said biological material with a chemical agent, wherein said biological material is adsorbed on a solid carrier and incubated with said chemical agent in the presence of an eluotropic salt corresponding to a NaCl concentration of at least 200 mM, incubation thereby being effected simultaneously with or immediately after elution of said biological material. - -

Applicants submit that the claims being added in the preliminary amendment are in compliance with all patentability requirements. Applicants therefore respectfully request that the claims be allowed. To expedite allowance, the Examiner is encouraged to contact Applicants' attorney at the number provided below.

Respectfully submitted,
Foley, Hoag & Eliot, LLP



Beth E. Arnold
Registration No. 35,430
Attorney for Applicant

Date: October 7, 1999
Patent Group
One Post Office Square
Boston, Massachusetts 02109
Telephone: (617) 832-1177
Facsimile: (617) 832-7000

A Method for Inactivating Pathogens, in Particular Viruses, in a Biological Material

The present invention relates to a method for inactivating pathogens in a biological material by incubation with a chemical agent.

A biological material is derived from organisms or body liquids or microorganisms.

Since a biological material may be contaminated with pathogens, such as, e.g., infectious molecules or microorganisms and viruses, and pyrogens, respectively, various methods for inactivating or depleting, respectively, pathogens and pyrogens, respectively, have already been developed.

Such methods include physical and/or chemical treatments, such as, e.g., diverse filtration methods (e.g. nano-, dia- or ultrafiltration), heat treatment, treatment with an acid or a base, treatment with a detergent and/or an organic solvent as well as treatment with UV light or with laser light. Also various combinations of such methods for inactivating and depleting, respectively, pathogens have been suggested in the prior art.

From EP 0 197 554, e.g., a method of depyrogenizing and inactivating viruses in a biological or pharmaceutical product is known, which comprises a treatment with a virus-inactivating and depyrogenizing

agent, such as, e.g., an amphiphilic substance and/or a solvent, on a solid phase on which the product has been adsorbed. After this treatment, the virus-inactivating and depyrogenizing agent is separated from the solid phase, the adsorbed product is washed and finally eluted from the solid phase.

From EP 0 131 740, the treatment of a protein-containing composition in a solution with organic solvents, such as di- or trialkyl phosphates, optionally in the presence of a detergent (solvent/detergent treatment) is known, whereby protein-compositions free from lipid-containing viruses can be obtained.

From AT patent 402,151, a heat treatment is known wherein to a preparation present in an aqueous solution a tenside is admixed at a concentration of at least 1 % by weight, prior to heating.

A further method for reducing or suppressing, respectively, undesired activities in biological or pharmaceutical products is known from EP 0 083 999. The latter is based on an extended contact with a solution or suspension of a non-denaturing amphiphile. The depyrogenized product is treated with an ion exchanger to remove the amphiphile.

A disadvantage of many of these methods known from the prior art is the frequent occurrence of losses of activity of the labile proteins, e.g. blood proteins,

contained in the compositions to be treated. In particular when carrying out a chromatographic purification step, inactivation of proteins occurs to a relatively large extent. A degradation of proteins may also lead to an activation. Thus it is, e.g., known that factor VII is very easily activated during a chromatographic purification due to autocatalytic processes to factor VIIa which factor is undesired because it is very labile.

A further disadvantage consists in the large amount of time and apparatus required for many methods, which greatly reduces their practicability and thus often makes their use unsuitable on a large-technical scale.

The present invention is based on the object of providing a method of effectively inactivating pathogens in biological materials, which method is protein-preserving, in particular labile blood proteins, which can be transferred easily onto a large-technical scale and can be carried out economically. In particular, in the method for inactivating pathogens, a degradation and a possible activation of proteins susceptible thereto is to be largely avoided.

The afore-mentioned object is achieved in that a method is provided for inactivating pathogens, in particular, viruses, in a biological material by incubation with a chemical agent, wherein the incubation is carried out in the presence of an

eluotropic salt corresponding to an NaCl concentration of at least 200 mmol/l, preferably at least 300 mmol/l.

Inactivation of pathogens in solution offers some advantages over the treatment of an adsorbent. Thus, e.g., the practicability of such a method in a homogenous, single-phase system is higher, and validation of the inactivation step is better possible. The better accessibility of pathogens in a relatively homogenous phase also seems to increase the efficiency of the method step.

The biological material preferably comprises a human protein and in particular is plasma or a plasma fraction or is derived from a cell culture. Preferably, the biological material comprises a blood factor, such as factor XII, XI, VIII, V, von Willebrand factor or fibrinogen, in particular a vitamin K-dependent protein, such as factor II, factor VII, factor IX, factor X, protein C, protein S or protein Z, respectively.

The proteins may be present as single factors, preferably in purified form, or in a complex mixture. In a very particularly preferred embodiment, the biological material comprises at least one factor of the prothrombin complex and, in particular, is a prothrombin complex-containing fraction or a factor VII-containing material, e.g. after cryoprecipitation of plasma, one departs from the corresponding

supernatant (cryosupernatant).

The preparation according to the invention preferably is one having FEIB activity (Factor Eight Inhibitor Bypassing Activity), i.e. a preparation which is suitable for treating factor VIII inhibitor patients.

The cell-culture-derived material preferably is a material comprising recombinantly prepared blood factors, among them factors of intrinsic or extrinsic coagulation, of fibrinolysis, of thrombolysis, or the inhibitors thereof, in particular vitamin K-dependent blood factors. As the cells, the cells commonly used for the expression of recombinant proteins are suitable, preferably mammalian cells, such as, e.g., Vero, CHO or BHK cells. The corresponding proteins may be subjected to the method of the invention for inactivation of possibly present pathogens either directly from the crude cell extract, it may, however, also be a pre-purified cell fraction.

The chemical agent is, e.g., a detergent (amphiphile, tenside), which preferably is contained in an amount of at least 1%, more preferred more than 5%, most preferred more than 10%; yet also other chemical agents may be employed according to the invention, in particular those of which a virucidal, bactericidal or depyrogenizing effect is already known, or mixtures of the most varying chemical agents.

The choice is, however, limited by the fact that the nativity of the biological material shall not be substantially adversely affected. For an economical mode of procedure, a chemical is chosen which retains more than 50% of the biological activity of the material, based on the activity prior to incubation, preferably at least 70%, in particular more than 85%. Retention of the biological activity means that the proteins contained in the biological material are able to fulfill the function or the various functions naturally ascribed to them. This biological activity may be determined and stated depending on the type of protein, e.g. by means of a standardized chromogenic test or by antigen determination.

Optionally, the chemical agent is separated after incubation.

By "detergent", generally a synthetic, organic, surface-active substance is to be understood.

Preferably, a non-ionic detergent is used in the method according to the invention. Non-ionic tensides, such as polyether, in particular alkyl phenol polyglycol ether, are i.a. products of ethoxylation of fatty acids, fatty acid amides, fatty amines, fatty alcohols, amine oxides, fatty acid esters of polyalcohols and sugar esters.

Such a tenside does not act denaturing on the proteins and preferably is selected from the group of

polysorbate and triton. As the polysorbate, e.g. Tween® is used.

If detergents are used as chemical agents, according to a preferred embodiment they are used without the addition of other agents, in particular without the addition of toxic organic substances or solvents, such as, e.g., TNBP. In this manner, a risk of contamination is minimized.

According to the method of the invention, the biological material is incubated with a chemical agent. Incubation means the contacting of the biological material with a solution, suspension or emulsion of a chemical agent for a period of time sufficiently long for inactivation of pathogens or pyrogenes, respectively, possibly present, at a specific temperature. Contacting may be simply effected by allowing the mixture to stand for a defined period of time.

Incubation is effected according to the present invention in the presence of an eluotropic salt. By "eluotropic salt" hereinafter the salt in mixture with chemical agent or the salt in a complex composition is to be understood, with the property of dissolving adsorbed substances out of solid or liquid-impregnated, also gel-type adsorbents and/or to displace them. Preferably, the eluotropic salt is a desorption agent as is used in chromatographic methods. The adsorbed

substance is i.a. sufficiently soluble in the presence of the eluotropic salt, i.e. preferably conditions are chosen which do not precipitate the biological material.

The type and concentration of the salt or of the composition, respectively, is generally selected depending on the adsorbent used. The eluting effect of a salt depends, e.g., on the polarity of the solvent, i.e. it increases e.g. in the sequence ethanol - acetone - methanol - water. The adsorbent may also be a solid phase, in particular a matrix suitable for ion exchange chromatography. In the composition containing the eluotropic salt, also further additives, e.g. further salts, may be contained. Preferably, the composition is an aqueous composition having a pH ranging between 6.0 and 8.0, preferably around 7.0.

In a preferred embodiment, sodium chloride is used as the eluotropic salt, yet also other alkaline or alkaline earth salts, among them $CaCl_2$, may be used. As the eluotropic salts, also so-called chaotropic agents, such as, e.g., urea, rhodanides or guanidinium, may be employed. The concentration of the salt is at least ≥ 200 mmol/l, preferably ≥ 300 mmol/l. The upper limit for the concentration employed will depend in particular on the solubility of the respective salt and, for NaCl, is e.g. around 2 mol/l. Chaotropic substances, such as, e.g., urea, may be employed

optionally even up to a concentration of 8 mol/l.

Incubation of the biological material with the chemical agent is effected for a period of time sufficiently long to inactivate pathogens possibly present, preferably for a period of between 10 min and 10 h, most preferred between 1 h and 5 h. The time required for the method according to the invention may be determined by means of model viruses, such as HIV, Sindbis, TBE or hepatitis viruses in a pre-assay.

Also the choice of temperature has an influence on the period of time to be employed. In the method of the invention, incubation preferably is carried out at room temperature, e.g. in a temperature range of between 15 and 45°C, in particular between 20 and 30°C.

In the method according to the invention, the biological material preferably is adsorbed on a solid carrier, purified, and incubation is effected immediately after elution of the purified material. Elution and incubation may be carried out consecutively, they may, however, also be effected simultaneously.

According to a further preferred embodiment, incubation is effected after a chromatographic purification of a biological material, the eluate having been still further processed, e.g. by centrifugation, filtration, or other physical methods.

Preferably, the solid carrier is a material

suitable for chromatography, in particular a material suitable for ion exchange chromatography, hydrophobic chromatography, or affinity chromatography. Materials, such as Sepharose®, Superdex®, Sephadex®, Spherodex®, Toyopearl®, or inorganic materials, such as hydroxyl apatite, are used.

As the ion exchanger, anion exchanger materials, such as, e.g., DEAE Sephacel®, DEAE-Sephadex®, DEAE-Sepharose® CL6B, DEAE-Sepharose® Fast Flow, QAE-Sephadex®, Q-Sepharose® Fast Flow, Q-Sepharose® High Performance, DEAE-Tris Acryl, DEAE Spherodex®, Q-Hyper-D (obtainable through Sepracor), DEAE-Toyopearl®, QAE-Toyopearl®, Fractogel® EMD-TMAE or other Fractogel materials may be used.

As examples of hydrophobic chromatographic materials, butyl-Sepharose®, octyl-Sepharose®, phenyl-Sepharose®, Fractogel®TSK-Butyl, t-Butyl-HIC Support or TSK Gel Butyl Toyopearl® ought to be mentioned.

The biological material may be directly adsorbed on the carrier from a complex mixture and purified, the inactivation step may, however, also be preceded or followed by further steps of purifying the material, further chromatographic purification steps being preferred within the scope of the present invention.

By the method according to the invention, pathogens are inactivated. By pathogens, also fragments of, e.g., viruses, in particular also the isolated genome or the

fragments thereof, are understood.

The pathogens may be lipid-enveloped pathogens, such as, e.g., hepatitis B virus, or non-lipid enveloped pathogens, such as, e.g., hepatitis A virus.

At present, virus inactivation methods are called effective if after applying the method to a sample of a biological material which had been admixed with a high dose of a test virus, e.g. HI virus or Sindbis virus as a model virus for hepatitis viruses, viruses can no longer be detected in the sample, and the virus titer thus has been reduced to below the detection limit. Detection and quantitation of nucleic acids may, e.g., be effected by means of a PCR method as described in AT patent 401,062, or by direct titration.

As a measure for inactivation, the so-called reduction factor is known which, after a single addition of test virus, is calculated from the decadic logarithm of the quotient of initial and final virus titers. From European Guideline EC III/8115/89-EN of the Commission of the European Communities, furthermore, the so-called total reduction factor is known. It is calculated from the sum of the reduction factors of individual, subsequent inactivation measures.

Preferably, a further, independent step for inactivating or depleting pathogens, respectively, is carried out. For this, all methods known from the prior

art are usable to minimize the risk of infection.

In particular, a filtration and/or a heat treatment is effected as a further step for inactivation or depletion, respectively.

As the filtration, preferably a nanofiltration is performed. A preferred heat treatment is carried out on the solid biological material, e.g. on a lyophilisate having a controlled water content, e.g. a water content of between 5 and 8%, and at a temperature of between 50 and 80°C, as is described in EP-0 159 311.

In a preferred embodiment, a 2-step treatment with a detergent as the chemical agent is provided. In doing so, a detergent is used in a first step in an amount of at least 1%, preferably at least 5%, most preferred at least 10%. In a second step, a further detergent is used in an amount of at least 10%, preferably at least 12%, most preferred at least 14%. The detergent used may be the same one for both steps; however, also different detergents may be used. Quite generally, the risk of a virus infection after administration of a corresponding preparation can be greatly reduced or eliminated, respectively, by the combination of steps for virus inactivation.

According to the present invention, also a chromatographically purified preparation is provided which comprises an autodynamically activatable blood factor having a portion of activated blood factor of

less than 50%, based on the content of activated and non-activated blood factor, preferably less than 40%, more preferred less than 30%, still more preferred less than 20%, further preferred less than 10%, most preferred less than 1%, and a detergent content.

In particular, the preparation is a prothrombin complex containing preparation having a factor VIIa activity of less than 50%, based on the content of activated and non-activated factor VII, preferably less than 10%, most preferred less than 1%. The detergent content of the preparation according to the invention is present in a pharmaceutically acceptable amount, preferably between 1% and the detection limit of the detergent.

By "autodynamically activatable blood factor", according to the present invention a blood factor is to be understood which is autocatalytically activatable by surface contact or by processes, such as, e.g., chromatographic processes. In particular, such a blood factor is a blood factor selected from the group of factor VII, factor XII, factor XI and pre-kallikrein.

In a further preferred embodiment, the preparation is free from serine protease inhibitors, such as, e.g., thrombin inhibitors, or co-factors, such as, e.g., heparin. In a special embodiment, the freedom from such substances exists already during a chromatographic process.

Therefore, the present invention also relates to corresponding preparations obtainable by the method according to the invention.

In the preparation according to the invention, also further additives may be contained, e.g. substances, such as amino acids, which act in a stabilizing manner.

The present invention shall be explained in more detail by way of the following examples without, however, being restricted thereto.

EXAMPLE 1:

Detergent treatment of activated prothrombin complex FEIBA in the presence of TWEEN®-80

15 mg of DEAE-Sephadex® A-50, from Pharmacia, were incubated for 15 min at room temperature with 1 ml of a solution of 30 g/l NaCl in water until swelling. Thereafter, the gel was separated from the swelling supernatant by centrifugation. There followed five washings of the gel with 1 ml of buffer each (9 g/l $Na_2HPO_4.2H_2O$, 7 g/l NaCl, pH 7.0) and two further washings with a buffer (7 g/l Na_3citrate.$2H_2O$, 7 g/l NaCl) also by resuspension and centrifugation.

30 ml of fresh frozen human citrated plasma were thawed at 0 to +4°C, and the cryoprecipitate incurred was separated by centrifugation at +2°C. The "cryosupernatant" resulting therefrom was incubated with the washed DEAE-Sephadex®, FEIBA being generated and adsorbed on the gel together with the factors of

the prothrombin complex and inert protein. Thereafter, coadsorbed inert protein was removed from the DEAE gel by washing with a buffer (9 g/l $Na_2HPO_4.2H_2O$, 7 g/l NaCl).

The buffer-moist gel/protein complex was then suspended for 1 h at 26°C with 1.5 ml of a solution of 150 mg/ml TWEEN®-80 and 30 mg/ml NaCl. By the treatment with the solution of high ionic strength, protein was desorbed together with the factors of the prothrombin complex and pathogens possibly present. Subsequently, the suspension was diluted by adding 6.5 ml of water and readsorbed for 1 h at room temperature, the protein fraction being readsorbed again, whereas components of the inactivated pathogen remained in solution together with the detergent. The gel/protein complex was then washed five times, each with 1 ml of a solution of 7 g/l NaCl in water so as to be detergent-free.

For elution, the gel was treated under stirring with 0.7 ml of a solution of 30 g/l NaCl in water. The eluate was then dialysed against distilled water, frozen, and lyophilized. After reconstitution of the lyophilisate, the FEIB-activity was determined according to AT-B 350 726.

A preparation of FEIBA prepared in the same manner, yet without treatment with a detergent, was used as the control.

The analysis of the preparation obtained exhibited

a specific activity of 3.2 U FEIBA/mg protein at a protein content of 16.6 mg/ml after reconstitution of the lyophilisate and was comparable with the method variant without detergent treatment, a specific activity of 2.8 U/mg protein being obtained at a protein concentration of 16.5 mg/ml.

EXAMPLE 2:

Detergent treatment at the desorption of FEIBA with extended incubation time

The prothrombin complex fraction was adsorbed on DEAE-Sephadex® analogous to Example 1, washed free from inert protein, subsequently it was desorbed with a TWEEN®/NaCl solution. However, the protein fraction was kept for 2 or 3 hours, respectively, in the desorbed state under otherwise equal conditions. Thereafter it was worked up to the final product as described in Example 1.

The analysis of these formulations yielded a specific activity of 2.5 U of FEIBA/mg of protein at a protein content of 16.6 mg/ml with 2 h of incubation in the presence of TWEEN®-80, and a specific activity of 2.3 U of FEIBA/mg of protein at a protein content of 17.4 mg/ml with 3 h of incubation with detergent.

Thus it could be demonstrated that also the extended contact time with the detergent was not connected with any substantial inactivation of the active substance or reduction of yield.

EXAMPLE 3:

Detergent treatment of FEIBA with readsorption on a different gel

FEIBA was prepared as described in Example 1. After the treatment and desorption with detergent, the solution obtained was transferred into a container in which 15 mg of DEAE-Sephadex® A-50, from Pharmacia, were pre-incubated to swelling in a solution of 30 g/l NaCl and subsequently were provided by five washings each with 1 ml of a buffer (9 g/l $Na_2HPO_4.2H_2O$, 7 g/l NaCl, pH 7.0), and two further washings with a buffer (7 g/l Na_3citrate.$2H_2O$, 7 g/l NaCl), each by re-suspension and centrifugation. After a 1 h adsorption of the diluted protein complex for separating the detergent, working up was effected according to the process described in Example 1. The thus obtained final product had a yield of 95% as compared to a FEIBA prepared according to the standard variant, i.e. without treatment with detergent, and was of comparable specific activity.

EXAMPLE 4:

Detergent treatment of activated prothrombin complex FEIBA in the presence of TWEEN®-80 at increased temperature

15 mg of DEAE-Sephadex® A-50, from Pharmacia, were incubated for 15 min at room temperature with 1 ml of a solution of 30 g/l NaCl in water until swelling.

Thereafter, the gel was separated from the swelling supernatant by centrifugation. There followed five washings of the gel with 1 ml of buffer each (9 g/l $Na_2HPO_4.2H_2O$, 7 g/l NaCl, pH 7.0) and two further washings with a buffer (7 g/l Na_3 citrate.$2H_2O$, 7 g/l NaCl) also by resuspension and centrifugation.

30 ml of fresh frozen human citrated plasma were thawed at 0 bis +4°C, and the cryoprecipitate incurred was separated by centrifugation at +2°C. The "cryo-supernatant" resulting therefrom was incubated with the washed DEAE-Sephadex®, FEIBA being generated and adsorbed on the gel together with the factors of the prothrombin complex and inert protein. Thereafter, coadsorbed inert protein was removed from the DEAE gel by washing with a buffer (9g/l $Na_2HPO_4.2H_2O$, 7 g/l NaCl).

The buffer-moist gel/protein complex was then suspended with 1.5 ml of a solution of 1 mg/ml TWEEN®-80 and 30 mg/ml NaCl for 1 h at room temperature, the protein fraction and non-specifically adsorbed impurities being desorbed. Subsequently, the gel was separated by filtration. By further addition of TWEEN®-80, the protein solution then was brought to a detergent concentration of 150 mg/ml, and subsequently was incubated either for 1 h at 26°C or for 1 h at 40°C with stirring so as to inactivate any pathogens possibly present. Thereafter, it was diluted by the addition of 6.5 ml of water, and a freshly washed

prepared DEAE-Sephadex® A-50 gel was readsorbed. Then it was washed detergent-free by five washings with 1 ml each of a solution of 7 g/l NaCl in water, and finally the preparation was further worked up as described in example 1.

The analysis of both variants of treatment at 26°C and at 40°C showed a specific activity of the FEIBA preparation comparable to that of a standard variant without virus inactivation. The yields were 75% of the standard variant.

EXAMPLE 5:

Detergent treatment of prothrombin complex in the presence of TWEEN®-80 (at present considered by Applicant to be the best mode of carrying out the invention)

30 ml of fresh frozen human citrated plasma were thawed at 0 to +4°C, and the cryoprecipitate incurred was separated by centrifugation at +2°C. The "cryo-supernatant" resulting therefrom was admixed with 2 IU of heparin/ml. Subsequently, the proteins of the prothrombin complex were adsorbed with DEAE-Sephadex® A-50 from Pharmacia, at a concentration of 0.5 mg/ml. The gel/protein complex was separated from the solution and washed each with a buffer 1 (4 g/l Na_3citrate.$2H_2O$, 7 g/l NaCl, 9 g/l $Na_2HPO_4.2H_2O$, 500 IU of heparin/l, pH 7.5) and subsequently washed with buffer 2 (4 g/l Na_3citrate.$2H_2O$, 7 g/l NaCl, 500 IU of heparin/l, pH

7.5).

The washed gel was then suspended for pathogen inactivation with 1.5 ml of a solution containing 150 mg of TWEEN®-80/ml and 30 mg of NaCl/ml, for 1 h at 26°C. By this treatment, the protein fraction was desorbed together with any pathogens or pathogen fragments possibly present, and in the course of incubation with the detergent, such pathogens were inactivated. Subsequently, it was diluted with 6 ml of water as described in example 1, and the protein fraction including the active substance was readsorbed to the ion exchange matrix for 1 h at room temperature. Then it was washed five times with 1 ml of a buffer (4 g/l Na_3 citrate, 7 g/l NaCl, 500 IU of heparin/l, pH 7.5) so as to be detergent-free, and eluted with a solution of 1 g/l Na_3 citrate.$2H_2O$, 30 g/l NaCl, 1,000 IU of heparin, pH 7.0. To the eluate, 1 IU of heparin/ml was admixed. The prothrombin complex-containing solution was rebuffered against a buffer containing 4 g/l Na_3 citrate.$2H_2O$, 8 g/l NaCl, pH 7.0, and lyophilized. In the reconstituted, lyophilized prothrombin complex the protein content and the content of prothrombin complex factors was tested; the results can be taken from Table 1.

A test mixture without TWEEN® treatment was prepared as the control. The analysis results can also be taken from Table 1.

TABLE 1

Comparison of the activities of the prothrombin complex factors after carrying out the method according to the invention and without that method

	Composition					
	Protein (mg/ml)	Prothrombin (U/ml)	Factor VII (U/ml)	Factor IX (U/ml)	Factor X (U/ml)	Protein C (U/ml)
Control	14.7	22.1	0.9	21.0	23.2	26.5
Preparation of invention	14.4	19.7	1.0	22.2	22.1	27.9

It has been shown that no substantial change of the composition of the prothrombin complex was effected by the detergent treatment.

EXAMPLE 6:

Detergent treatment of factor VII with TWEEN®-80 as compared to virus inactivation of factor VII according to a conventional method

From human citrated plasma, the prothrombin complex fraction containing the coagulation factors prothrombin, slight portions of factor VII, factor IX and factor X were separated as described in example 5. The major portion of coagulation factor VII remaining in the supernatant after adsorption on DEAE Sephadex® A-50 was then recovered by adsorption on aluminum hydroxide. To this end, 10 ml of a 2% aluminum hydrogel suspension were admixed per 1 l supernatant after separation of the prothrombin complex and stirred at 4°C for 30 min. Subsequently, the aluminum hydroxide/protein complex was separated by centrifugation at 5,000 rpm for 10 min at approximately 4°C in a Sorvall RC3B rotor H6000A, the supernatant was discarded, and the precipitate was suspended with 3.5 % of the volume of the prothrombin complex supernatant used for adsorption, in a solution of 4 g/l of Na_3 citrate.$2H_2O$ and 7 g/l of NaCl, pH 7.5, and stirred for 30 min. By this, inert protein was desorbed from the aluminum hydroxide. The factor VII remaining on the

aluminum hydroxide was pelletized by renewed centrifugation as described above. The supernatant was discarded, and the precipitate was further used for further processing. For desorption of the protein fraction, the aluminum hydroxide/factor VII complex was stirred for 30 min with 1 % by volume of the prothrombin complex supernatant of a 0.3 mol/l phosphate buffer, pH 8.6 (53.4 g/l of $Na_2HPO_4.2H_2O$ were adjusted to pH 8.6 with a solution of 41.1 g/l $NaH_2PO_4H_2O$) used for adsorption and containing 1 % of TWEEN®-80. Subsequently, for pathogen inactivation, detergent was added to a final concentration of 15 % of TWEEN®-80, and then it was stirred for 1 h at 40°C. Thereafter, the solution was cooled to approximately 22°C and diluted with 9 parts of aqua dest.. The factor VII fraction was then readsorbed on 1 g/l DEAE-Sephadex® A-50 under stirring for 1 h at approximately 22°C. Then the gel/protein complex was washed detergent-free on the sintered suction filter by washing three times, with 100 ml each per liter of employed, diluted TWEEN® solution, with a buffer containing 4 g/l Na_3 citrate.$2H_2O$ and 7 g NaCl/l, pH 7.5, containing 500 IU of heparin/l. The elution of the factor VII fraction was effected by stirring of the ion exchanger protein complex and 100 ml/l of diluted TWEEN® solution of a 85 g/l NaCl containing solution for 30 min at 22°C. In the eluate, subsequently the

factor VII content was measured by means of a chromogenic factor VII test, (Immunochrom Faktor VII:C, IMMUNO AG, Vienna, measured against the international prothrombin complex standard), the protein content was quantitated according to the method of Bradford [Anal. Biochem. 72:248-254 (1976)] and factor VIIa according to the method from US 683,682 (measured against the international factor VIIa standard). The results can be taken from Table 2.

For a comparison, factor VII was separated from the other proteins of the prothrombin complex by adsorption on aluminum hydroxide, as described above, and in the adsorbed state it was treated according to EP 0 197 554 with the virus-inactivating agents from EP 0 131 740 with TWEEN®-80 and tri-(N-butyl)-phosphate (TNBP). To this end, the alhydrogel protein complex was stirred in an aqueous solution of 1% TWEEN®-80 and 0.3% tri-(N-butyl)-phosphate for 18 h at 4°C with a volume of 50 ml/l prothrombin complex supernatant. Subsequently it was centrifuged as described above to separate the aluminum hydroxide protein complex, and by washing with 3 x 100 ml of a solution of 4 g/l Na_3 citrate.$2H_2O$, 7 g/l NaCl, pH 7.5, it was freed from an excess of TWEEN®-80 and tri-(N-butyl)-phosphate by resuspending. Between each wash, there followed a pelletizing of the aluminum hydroxide/protein complex by centrifugation. Elution was carried out under the same conditions as in

the parallel test mixture according to the method of the invention. Likewise, the analyses of the final product were carried out analogously. The results can be taken from Table 2.

TABLE 2

Factor VIIa activities after carrying out the method of the invention and after carrying out the method according to EP 0 197 554.

	Composition				
	FVII-activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)	Factor VIIa activity (U/ml)	(VIIa/VII)
Preparation of invention	3.2	0.2	15.2	2.7	0.84
Preparation acc. to EP 0197 554/ EP 0131 740	3.8	0.5	7.6	11.9	3.13

It has been shown that by applying this method, the factor VIIa content was markedly increased as compared to the method according to the invention, yet despite the complex treatment of factor VII no activation could be found. Moreover, with the method according to the invention, the specific activity of the obtained product was higher than in the comparative preparation.

EXAMPLE 7:

Semiquantitative determination of hepatitis G virus

In the pathogen inactivation formulations of examples 1 to 6, the samples were drawn from each of the starting materials, supernatant after cryoprecipitation or adsorption supernatant after separation of the coagulation factors II, IX and X, as well as the correspondingly purified and concentrated coagulation factor preparations. 0.5 ml of these samples were diluted 1 + 1 with physiological phosphate-saline buffer, and viruses possibly present were pelletized by ultracentrifugation. The RNA was extracted from the viral pellets by means of the RNAzol reagent method (Biotecx, Houston, Texas), and dissolved in sterile a. dest..

RT-PCR for hepatitis G virus (HGV)nucleic acids was carried out with the primer pair NS5a 1 and NS5a 2 (Linnen, J. et al., Science 271: 505-508 (1996)). The sequence of the primer used (obtainable from Boehringer Mannheim, Germany) for NS5a 1 was:

5'CTCTTTGTGGTAGTAGCCGAGAGAT 3', and for NS5a 2: 5'CGAATGAGTCAGAGGACGGGGTAT 3'. The primers were labelled with a fluorescent dye, and the fluorescent amplicons resulting therefrom according to the routine methods of common PCR protocols were analyzed on an ABI 377-Sequencer of Applied Biosystems. In order to be able to exclude the presence of RT-PCR inhibitors in the samples, the samples were spiked with hepatitis C virus-RNA mimics and analyzed in a hepatitis C-PCR carried out according to EP 0 714 988. Exclusively extracts which did not show any inhibition in the HCV-PCR were used as evaluatable for HGV-PCR. The intensity of the fluorescence was taken as a measure for the content of hepatitis G virus. It has been shown that starting materials used for fractionation had highly positive signals prior to pathogen inactivation according to the inventive method, i.e. had a high concentration of HGV nucleic acid amplificates, whereas in the eluates after readsortpion and separation of the virus-inactivating agents, no HGV-RNA could be detected any longer.

In parallel assays without carrying out a detergent treatment, the eluates as well as the starting materials used were HGV-PCR-positive.

C l a i m s :

1. A method for inactivating pathogens, in particular viruses, in a biological material by incubation with a chemical agent, characterized in that the biological material is adsorbed on a solid carrier and incubation is carried out with a chemcial agent in the presence of an eluotropic salt corresponding to a NaCl concentration of at least 200 mM, preferably at least 300 mM, whereby incubation is effected simultaneously with the elution or immediately after the elution of the biological material.

2. A method according to claim 1, characterized in that a detergent is used as the chemical agent, which is preferably contained in an amount of at least 1%, more preferred more than 5%, most preferred more than 10%.

3. A method according to claim 1 or 2, characterized in that sodium chloride is used as the eluotropic salt.

4. A method according to any one of claims 1 to 3, characterized in that the incubation is carried out for

a period of time of between 10 min and 10 h, most preferred between 1 h and 5 h.

5. A method according to any one of claims 1 to 4, characterized in that plasma or a plasma fraction or material from a cell culture is used as the biological material.

6. A method according to any one of claims 1 to 5, characterized in that a biological material is used which comprises a blood factor, in particular a vitamin K-dependent protein.

7. A method according to any one of claims 1 to 6, characterized in that a biological material is used which is a prothrombin complex-containing fraction.

8. A method according to any one of claims 1 to 7, characterized in that the biological material is adsorbed on a solid carrier, is purified, and incubation is carried out after elution of the purified

A Method for Inactivating Pathogens, in Particular Viruses, in a Biological Material

A b s t r a c t :

A method of inactivating pathogens, in particular viruses, in a biological material by incubation with a chemical agent, which method is characterized in that incubation is carried out in the presence of a eluotropic salt corresponding to a NaCl concentration of at least 200 mmol/l, preferably at least 300 mmol/l, as well as a chromatographically purified, prothrombin complex containing preparation are disclosed.

DECLARATION FOR PATENT APPLICATION Docket Number: BHV-313.01

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR INACTIVATING PATHOGENS, ESPECIALLY VIRUSES, IN A BIOLOGICAL MATERIAL

the specification of which (check one): ( ) is attached hereto.
(X) was filed on 04/06/98 as ~~United States~~ International Patent Application Number PCT/AT98/00090 and was amended on 03/04/99 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulation, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed
A 594/97 (Number)	Austria (Country)	08/April/1997 (Day/Month/Year Filed)	(X) Yes ( ) No
(Number)	(Country)	(Day/Month/Year Filed)	( ) Yes ( ) No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States Provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

PCT/AT98/00090 (Application Number)	April 6, 1998 (Filing Date)	(Status: patent, pending, abandoned)
(Application Number)	(Filing Date)	(Status: patent, pending, abandoned)

I hereby appoint Beth E. Arnold, Reg. No. 35,430; Paula Campbell, Reg. No. 32,503, Charles H. Cella, Reg. No. 38,099; Isabelle M. Clauss, Reg. *(see attached)*; Edward J. Kelly, Reg. No. 38,936; Donald W. Muirhead, Reg. No. 33,978; Chinh Pham, Reg. No. 39,329; Diana Steel, Reg. No. 43,153, Matthew P. Vincent, Reg. No. 36,709; and Anita Varma, Reg. No. 43,221 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Address all telephone calls to Beth E . Arnold at telephone number (617) 832-1000.

Address all correspondence to: Patent Group
Foley, Hoag & Eliot LLP
One Post Office Square
Boston, MA 02109-2170

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor (given name, family name): Hans-Peter SCHWARZ

Inventor's signature — 30/November/1999 Date

Schindlergasse 32, A-1180 Vienna, Austria AT — Austria
Residence — Citizenship

Schindlergasse 32, A-1180 Vienna, Austria
Post Office Address (if different)

Full name of second or joint inventor, if any, (given name, family name): Gerold ZERLAUTH

Inventor's signature — 30/November/1999 Date

Sobieskigasse 42/9, A-1090 Vienna, Austria AT — Austria
Residence — Citizenship

Sobieskigasse 42/9, A-1090 Vienna, Austria
Post Office Address (if different)

Full name of third inventor (given name, family name): Peter TURECEK

Inventor's signature — 30/November/1999 Date

Hauptstrasse 59g, Weidling, A-3400 Klosterneuburg, Austria — Austria
Residence — Citizenship

Hauptstrasse 59g, Weidling, A-3400 Klosterneuburg, Austria
Post Office Address (if different)